

1 WHAT IS CLAIMED IS:

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- 3 1. A computer implemented method of localizing a biomarker within a cell,
- 4 comprising:
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- 6 identifying portions of a first image of the cell that corresponds to a first
- 7 defined area;
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- 9 identifying portions of a second image of the cell that corresponds to at least
- 10 one biomarker;
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- 12 determining portions of the second image that lie within the portions of the
- 13 first image to identify whether the biomarker is localized within the defined
- 14 area.
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- 16 2. The method of claim 1, wherein the portions of the first image and portions of the
- 17 second image comprise pixels.
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- 19 3. The method of claim 1, wherein the portions of the first image comprise pixels
- 20 having an intensity associated with the first defined area of the cell.
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- 22 4. The method of claim 3, further comprising determining the intensity associated
- 23 with the first defined area of the cell.
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- 25 5. The method of claim 2, wherein the portions of the second image comprise pixels
- 26 having an intensity corresponding to the at least one biomarker.
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- 28 6. The method of claim 5, further comprising determining the intensity associated
- 29 with the at least one biomarker.
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31 7. The method of claim 1, further comprising reducing representation of out-of-focus  
32 elements in the first image.

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34 8. The method of claim 7, wherein reducing representation comprises manipulating  
35 image pixel intensities of the first image based on image pixel intensities of a third  
36 image featuring a different depth of focus.

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38 9. The method of claim 1, wherein the defined area is selected from the group  
39 consisting of the cell nucleus, cytoplasm, nuclear membrane, cellular membrane,  
40 mitochondria, endoplasmic reticulum, peroxisome and lysosome.

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10. The method of claim 1, wherein the cellular component is selected from the  
group consisting of a protein, peptide, nucleic acid, lipid or carbohydrate.

11. A method of analyzing a cell containing sample, comprising:

obtaining a first image of a cell with a first stain that is selective for a first  
defined area within the cell and a second stain that is selective for at least one  
biomarker;

determining an intensity value for the first stain at a plurality of pixel  
locations in the first image;

based on the intensity values, determining pixel locations in the first image  
that correspond to the first defined area within the cell and assigning  
those pixel locations to the first defined area;

obtaining a second image and determining an intensity value for the second  
stain at a plurality of pixel locations in the second image; and

comparing the first and second images to identify pixel locations in the second  
image that are within the first cellular compartment.

- 60 12. The method of claim 11, wherein the defined area is selected from the group  
61 consisting of the cell nucleus, cytoplasm, nuclear membrane, cellular membrane,  
62 mitochondria, endoplasmic reticulum, peroxisome and lysosome.
- 63 13. The method of claim 11, wherein the cellular component is selected from the  
64 group consisting of a protein, peptide, nucleic acid, lipid or carbohydrate.
- 65 14. The method of claim 11, wherein the cell is contacted with a third stain that  
66 is selective for a second defined area within the cell, the method further  
67 comprising:
- 68 in a third image of the distribution of the third stain in the cell, determining an  
69 intensity value for each pixel location of a plurality of pixel locations in the  
70 third image;
- 71 based on the intensity values, determining which pixel locations in the third  
72 image correspond to the second defined area of the cell and assigning those  
73 pixel locations to the second defined area; and
- 74 identifying which of the pixel locations in the second image are within the  
75 second defined area.
- 76 15. The method of claim 14, further comprising assigning pixel locations not  
77 assigned to the first or second defined areas to a third defined area, and identifying  
78 which of the pixel locations in the second image are within the third defined area.
- 79 16. The method of claim 15, wherein the first, second, and third defined areas are  
80 selected from the group consisting of: a nucleus, cytoplasm, nuclear membrane,  
81 cellular membrane, mitochondria, endoplasmic reticulum, peroxisome and lysosome.
- 82 17. The method of claim 14, wherein the cell is contacted with a fourth stain that  
83 is selective for a defined area in the cell, and at least one pixelated image of the  
84 distribution of the fourth stain is acquired, the method further comprising

85 reading a third intensity value for each of a plurality of pixels in the image of  
86 the fourth stain distribution;

87 determining a threshold intensity value from the third intensity values;

88 comparing the third intensity value for each of the plurality of pixels to the  
89 threshold intensity; and

90 assigning pixel locations to a mask based on the threshold intensity value.

91 18. The method of claim 17, wherein the pixel locations in the plurality of pixels in  
92 the image of the first stain distribution are the pixel locations in the mask set.

93 19. The method of claim 18, wherein the pixel locations assigned to the mask  
94 comprise the location of pixels having third intensity values equal to or greater than  
95 the threshold intensity value.

96 20. The method of claim 19, further comprising binning the third intensity values for  
97 each of the plurality of pixels in the image of the fourth stain distribution.

98 21. The method of claim 20, wherein the threshold intensity value is determined from  
99 an intensity value of a largest bin.

100 22. The method of claim 20, wherein the threshold intensity value is determined from  
101 an intensity value of a second largest bin.

102 23. The method of claim 17, further comprising comparing for each pixel location  
103 the first intensity value to the second intensity value and assigning the pixel location  
104 to the second defined area when the second intensity value is greater than the  
105 first intensity value.

106 24. The method of claim 17, further comprising reading a signal intensity value for  
107 each pixel location in an array of pixels in the image of the second stain distribution,  
108 and summing the signal intensity values to determine a total signal intensity.

109 25. The method of claim 17, wherein the array of pixels in the image of the second  
110 stain distribution is the first defined area in the cell.

111 26. The method of claim 17, further comprising reading a signal intensity value for  
112 each pixel location in an array of pixels in the image of the second stain distribution,  
113 and summing the signal intensity values.

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115 27. The method of claim 17, wherein the array of pixels in the image of the second  
116 stain distribution is the second defined area.

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118 28. The method of claim 17, further comprising reading a signal intensity value for  
119 each pixel location in an array of pixels in the image of the second stain distribution,  
120 and summing the signal intensity values to determine a total intensity.

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122 29. The method of claim 17, wherein the array of pixels in the image of the second  
123 stain distribution is the third defined area.

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125 30. A method of analyzing a cell containing sample, comprising:

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127       accessing a value for each of a plurality of pixel locations in a first image of a  
128       cell;

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130       accessing a value for each of the plurality of pixel locations in a second  
131       image of a cell; and

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133       subtracting a percentage of the intensity value for each pixel location in the  
134       second image from the intensity value of the same pixel location in the first  
135       image to obtain an adjusted intensity value.

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137 31. The method of claim 30, wherein the first image is acquired at a first focal plane  
138 and the second image is acquired at a second focal plane.

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140 32. The method of claim 30, wherein the percentage of the intensity value for each  
141 pixel location in the second image subtracted is determined from an intensity  
142 distribution function.

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144 33. A method of analyzing a plurality of spots, comprising:

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146 locating the spots;

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148 defining a reference point for the located spot;

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150 connecting the reference point of the located spot to the reference point of a  
151 set of nearest neighbor spots with a corresponding line segment; and

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153 identifying points of intersection between line segments used to connect spots  
154 to each other.

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156 34. The method of claim 33, further comprising assigning reference points and points  
157 of intersection a reference number.

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159 35. The method of claim 34, further comprising tabulating the reference numbers and  
160 spot locations.

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162 36. The method of claim 33, wherein the reference point within each spot is the  
163 center of the spot.

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165 37. The method of claim 33, wherein the spots are histospots in a tissue microarray.

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167 38. The method of claim 33, further comprising connecting each of a plurality of  
168 reference points to a nearest edge using a corresponding line segment.

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